Jones et al., http://www.jcb.org/cgi/content/full/jcb.201401045/DC1

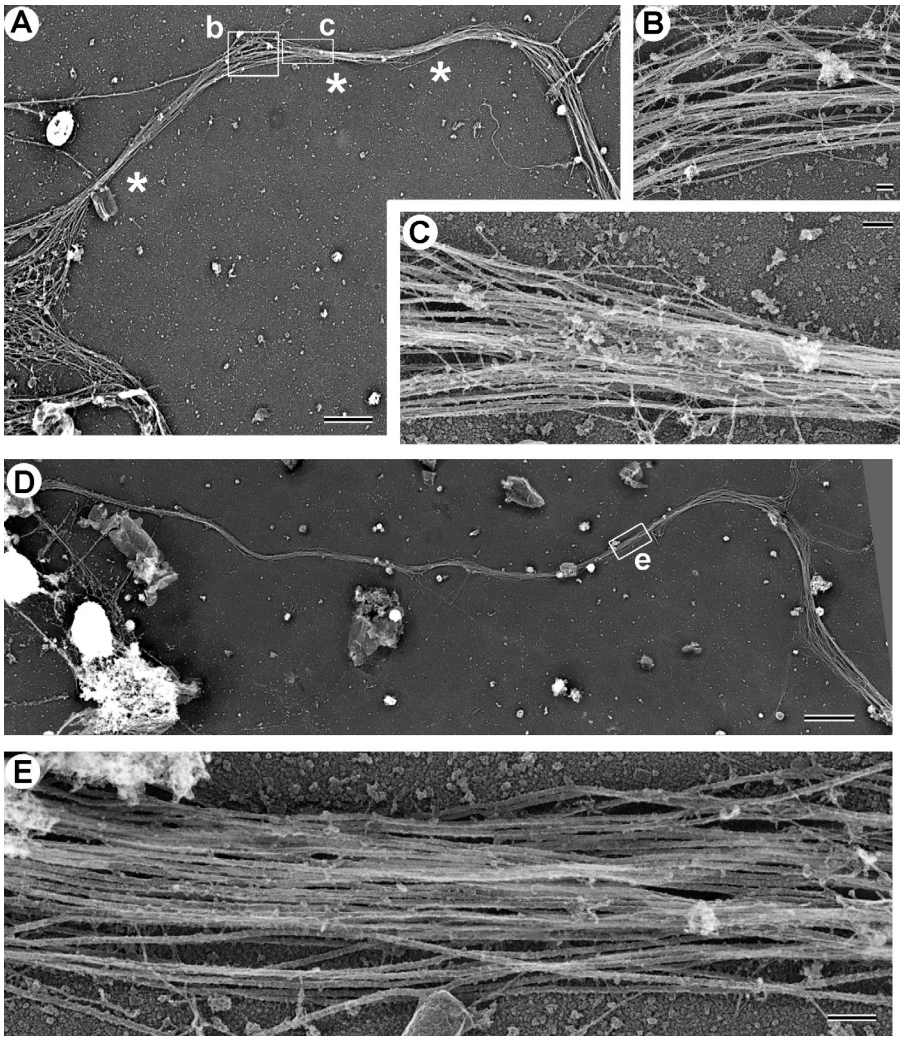

Figure S1. **Microtubule bundling in the proximal axon of DIV3 neurons.** (A–C) PREM of an axon with intermittent bundling of microtubules. (A) Low-magnification image of the proximal axon. Bundled regions are marked by asterisks. (B and C) Zoomed boxed regions with corresponding letters from A showing loosely aligned microtubules (B) and the boundary between regions with loosely aligned and bundled microtubules (C). (D and E) Continuous bundling of microtubules. (D) Low-magnification image of the proximal axon. (E) Zoomed boxed region from D showing bundled microtubules. Bars: (A) 1 µm; (B and C) 200 nm; (D) 5 µm; (E) 500 nm.

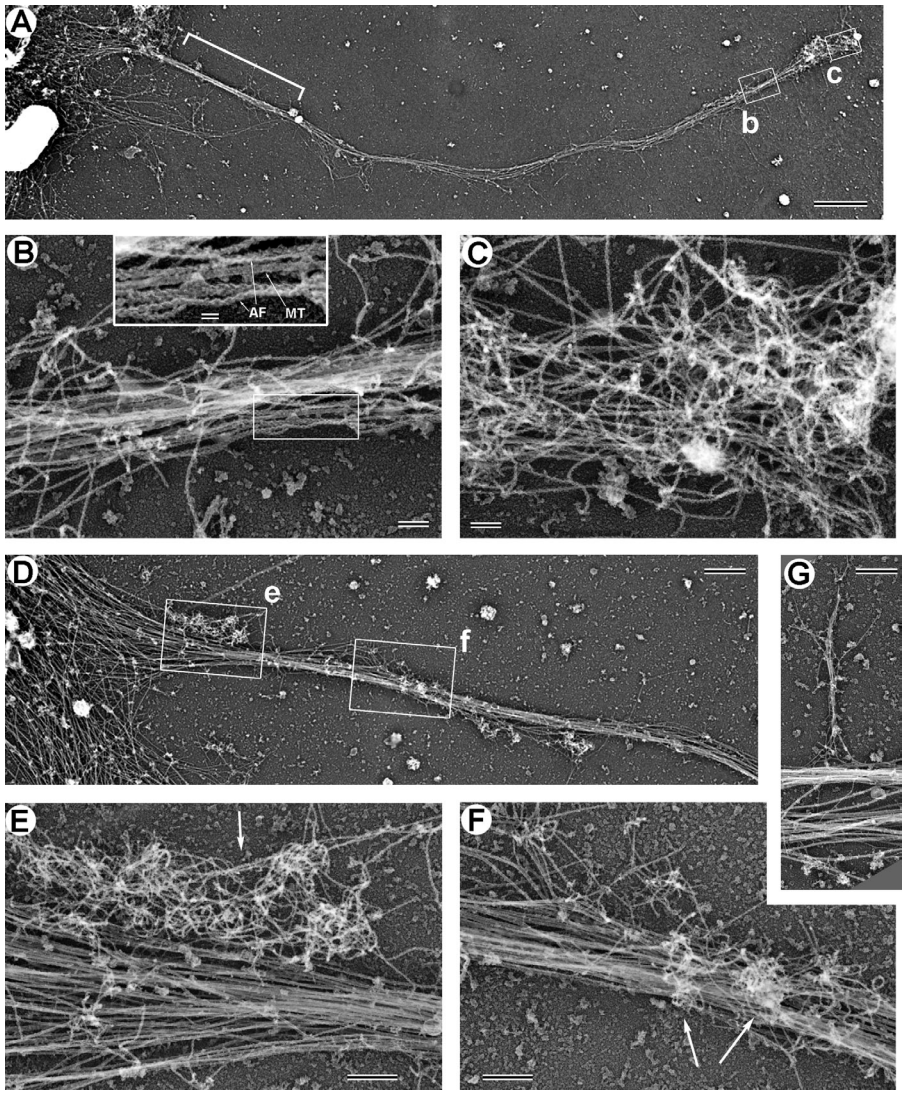

Figure S2. **Actin structures in axons of DIV3 neurons decorated with S1.** (A–C) PREM of the distal axon. (A) Low-magnification image of the axon. Cell body is at left. Note tight packing of microtubules in the proximal region (bracket) compared with their loose arrangement in the rest of the axon. (B and C) Zoomed boxed regions with corresponding letters from A showing enrichment of actin filaments in the distal axon shaft (B) and the growth cone (C). Inset in B shows S1-decorated actin filaments (AF) and a microtubule (MT) enlarged from the boxed region in B. (D–F) Proximal axon. (D) Low-magnification image of a proximal region of the axon. Cell body is at left. (E and F) Zoomed boxed regions with corresponding letters from D showing enrichment of actin filaments in lateral (E) or dorsal (F) actin patches (arrows) associated with an array of microtubules. (G) Filopodium in a midsection of an axon. Bars: (A) 5 µm; (B and C) 200 nm; (B, inset) 50 nm; (D) 2 µm; (E–G) 500 nm.

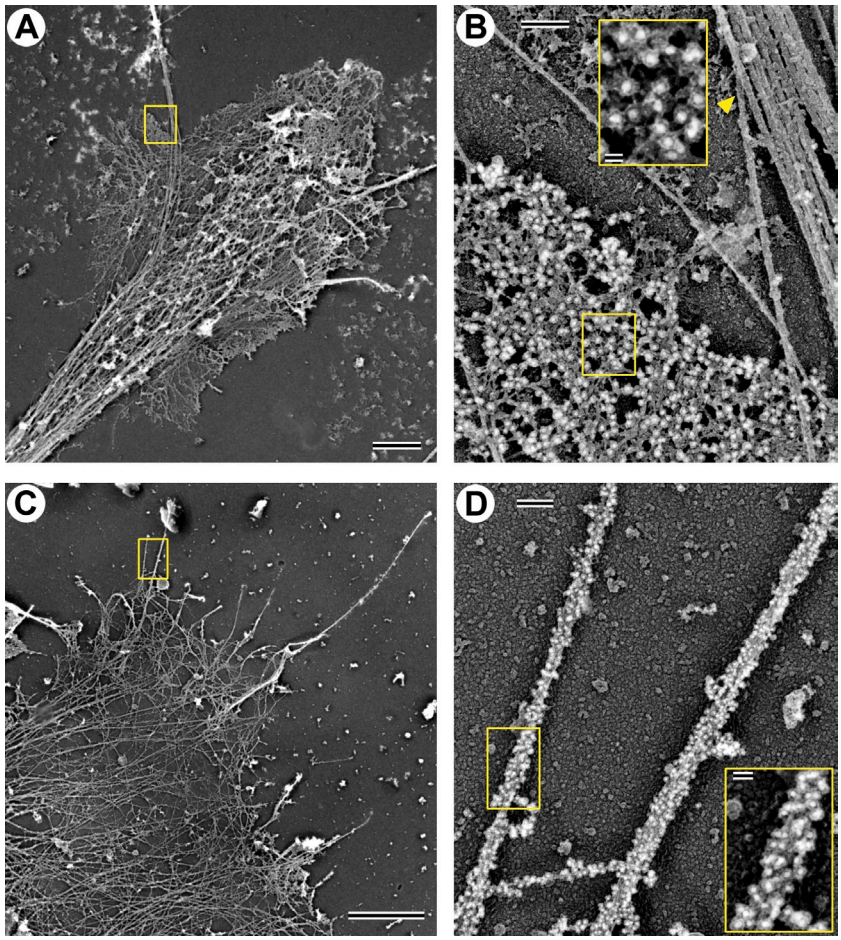

Figure S3. **PREM of immunogold-labeled actin structures in neuronal cultures.** A neuronal growth cone (A and B) and nonneuronal cell (C and D) from a DIV10 hippocampal neuronal culture were stained with Alexa Fluor 488–phalloidin, followed by primary Alexa Fluor 488 antibody and secondary 18-nm gold-conjugated antibody. Boxed regions from low-magnification images A and C are enlarged in B and D, respectively, and further enlarged in insets to show phalloidin immunogold-labeled actin filaments and unlabeled microtubules (arrowhead) in the growth cone leading edge (B) and labeled actin filaments in filopodia of a nonneuronal cell (D). Bars: (A) 2.5 µm; (B and D) 200 nm; (B, inset) 50 nm; (D, inset) 30 nm; (C) 5 µm.

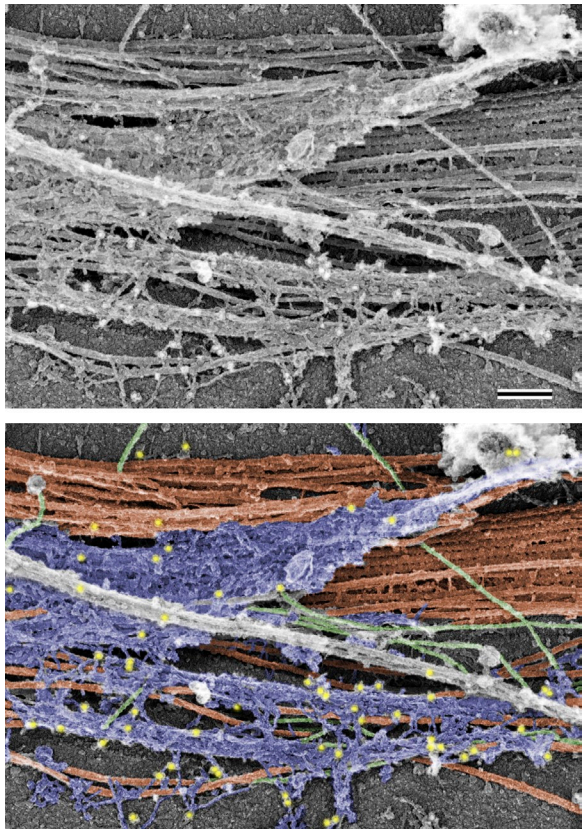

Figure S4. **The AIS coat breaks and detaches from microtubules after LatB treatment.** (top) PREM of a DIV10 hippocampal AIS showing intact portions of the coat (left) and the underlying microtubules bundles in regions where the coat is detached (right). (bottom) A duplicate image of the top panel with pseudocoloring marking AIS coat (purple), microtubules (red), neurofilaments (green), and phalloidin immunogold (yellow). Notice part of the AIS coat appears torn away. Bar, 200 nm.